RESEARCH ARTICLE

Dopamine D1 Receptors Regulate Spines in Striatal Direct-Pathway and Indirect-Pathway Neurons

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ABSTRACT: Background: Dopamine transmission is involved in the maintenance of the structural plasticity of direct-pathway and indirect-pathway striatal projection neurons (d-SPNs and i-SPNs, respectively). The lack of dopamine in Parkinson's disease produces synaptic remodeling in both types of SPNs, reducing the length of the dendritic arbor and spine density and increasing the intrinsic excitability. Meanwhile, the elevation of dopamine levels by levodopa recovers these alterations selectively in i-SPNs. However, little is known about the specific role of the D1 receptor (D1R) in these alterations. **Methods:** To explore the specific role of D1R in the synaptic remodeling of SPNs, we used knockout D1R mice ($D1R^{-/-}$) and wild-type mice crossed with drd2-enhanced green fluorescent protein (eGFP) to identify d-SPNs and i-SPNs. Corticostriatal slices were used for reconstruction of the dendritic arbors after Lucifer yellow intracellular injection and for whole-cell recordings in naïve and parkinsonian mice treated with saline or levodopa.
Results: The genetic inactivation of D1R reduces the length of the dendritic tree and the spine density in all SPNs, although more so in d-SPNs, which also increases their spiking. In parkinsonian $D1R^{-/-}$ mice, the spine density decreases in i-SPNs, and this spine loss recovers after chronic levodopa.
Conclusions: D1R is essential for the maintenance of spine plasticity in d-SPNs but also affects i-SPNs, indicating an important crosstalk between these 2 types of neurons. © 2020 International Parkinson and Movement Disorder Society

Key Words: dopamine; Parkinson's disease; spines; striatum; synaptic plasticity

The striatum is a key component of the basal ganglia, playing an important role in the control of motor behavior.[1] This nucleus is mainly composed of direct-pathway striatal projection neurons (d-SPNs) and indirect-pathway striatal projection neurons (i-SPNs), expressing dopamine D1 and D2 receptors, respectively.[2] The striatum is densely innervated by dopaminergic inputs from the substantia nigra pars compacta and ventral tegmental area and glutamatergic inputs from the cortex and thalamus.[2] Dopaminergic afferents synapse onto SPNs, and the released dopamine activates dopamine receptors.[3] D1R and D2R are colocalized with glutamate receptors in the dendritic spines and are critical for SPNs activity.[4]

Dopamine signaling in the striatum is implicated in modulating synaptic plasticity in SPNs.[5] The lack of dopamine in Parkinson's disease (PD) produces a profound transformation in the basal ganglia circuits, resulting in the loss of dendritic spines in SPNs[6-15] and an increase in SPNs excitability[10,12,13,16,17] as a homeostatic mechanism to maintain global activity. Administration of the dopamine precursor levodopa (L-dopa) after dopamine depletion selectively remodels i-SPNs without affecting d-SPNs.[10-12] However, chronic L-dopa treatment induces dyskinesia, which is related to D1R hypersensitization.[15,18-23]

High synaptic dopamine levels, such as those induced by cocaine and amphetamines, increases the number of dendritic spines and produces long-term adaptations in SPN plasticity.[24] During development, dopamine also modulates spinogenesis and excitability in SPNs,[25,26] and D1-like receptor agonists increase corticostriatal activity and spine density in d-SPNs in a protein kinase

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A-dependent manner.[27] Interestingly, dopamine promotes spine enlargement in d-SPNs by D1R activation.[28] However, the specific role of D1R in the synaptic remodeling and spine profile of d-SPNs and i-SPNs remains unknown. To establish the specific role of D1R, we used D1R knockout mice ($D1R^{-/-}$) crossed with bacterial artificial chromosome-drd2-eGFP mice to identify the SPNs. Although hyperactive, $D1R^{-/-}$ mice exhibited impaired motor coordination and slightly reduced basal dopamine.[29-34] D1R inactivation blocks hippocampal and corticostriatal long-term potentiation and FosB induced by D1R agonists,[19,35-37] suggesting that striatal function is altered in $D1R^{-/-}$ mice.

We found that D1R inactivation reduces the neuronal architecture and increases the intrinsic excitability of d-SPNs, as with dopamine depletion. Strikingly, D1R inactivation induces a small but consistent spine density reduction in i-SPNs, which is potentiated after dopamine depletion. We show that D1R has a critical role in spine density and the intrinsic excitability of d-SPNs but is also involved in i-SPN spine density.

Methods and Materials

Four-month-old or 5-month-old male and female hemizygous BAC-transgenic mice (D2R-eGFP, C57BL/6; wild type [WT] group) and $D1R^{-/-}$ D2R-eGFP mice ($D1R^{-/-}$ group) were used. No sexual differences were observed within any group, so the results were pooled. All animal procedures were approved by the CSIC Ethical Committee and followed European Union Guidelines (2010/63/EU).

Striatal Lesion and Treatments

The 6-hydroxydopamine (6-OHDA) lesions and L-dopa treatment were performed as described.[11,12,19,38] [11,12,19,38] Mice were sacrificed 1 hour after the last L-dopa or saline injection. Some WT mice were treated with reserpine (5 mg/kg) on 2 consecutive days (reserpine group; Supplementary Table 1).

Single-Cell Microinjection and Spine Analysis

The mice were anesthetized with pentobarbital (1 ml/kg) and perfused fixed with 4% paraformaldehyde. For SPN morphological reconstructions, we used 200 μm sections. Identified SPNs were filled with 8% Lucifer yellow (Sigma-Aldrich, Madrid, Spain) and injected with a 10 to 20 nA hyperpolarizing current. The d-SPNs (GFP negative) and i-SPNs (GFP positive) located in the dorsolateral striatum (Supplementary Fig. S1) were filled with Lucifer yellow, and 2-dimensional representation of the complexity of the 3-dimensional dendrite tree were analyzed using Neurolucida v8 software (MicroBrightField, Williston, VT).[11-13] We analyzed 4 to 9 SPNs per animal (Supplementary Table 1). Only completely filled neurons were analyzed. Filled neurons near the lesion track were discarded. For spine density analysis, we quantified the total spine number in 1 dendrite per SPN, usually one expanded in a horizontal plane (x-axis). The researcher was blinded to the experimental conditions.

Whole-Cell Patch Clamp Recordings

The mice (Supplementary Table 2) were anesthetized as noted previously and transcardially perfused with ice-cold N-methyl-D-glucamine-HEPES. Coronal brain slices (275 μm) were incubated for 60 minutes with a recovery solution at room temperature before recording with standard artificial cerebrospinal fluid solution at 31 to 32°C. Identified dorsal striatum SPNs were recorded with patch pipettes (5–8 MΩ) filled with intracellular solution. Current-clamp (for intrinsic properties and firing rate experiments) or voltage-clamp (for excitatory postsynaptic currents [EPSCs]) recordings were performed as described (Supplementary Material).

Statistical Analysis

Data are expressed as mean per animal ± standard error of the mean. Statistical evaluations and graphs were generated using SigmaPlot 12.0 (Systat-Software Inc., San Jose, CA). Statistical differences ($P < 0.05$) were assessed by t test and 1-way or 2-way analysis of variance (ANOVA) followed by Bonferroni t test or Kruskal-Wallis analysis followed by Dunn's test for nonparametric data.

Results

The severe lack of striatal dopamine increases the excitability and reduces the spine density in both types of SPNs.[11-13] Other authors observed increased excitability in d-SNPs[16,17] and a decrease in i-SPNs,[10] indicating that dopamine is important for the synaptic regulation of SPNs; however, the precise role of D1R or D2R in these processes is not well established. To evaluate the role of D1R in the activity and morphology of SPNs, we used $D1R^{-/-}$ mice crossed with BAC-D2R-eGFP mice to identify d-SPNs (negative-eGFP) and i-SPNs (positive-eGFP) compared with BAC-transgenic WT ($D1R^{+/+}$) mice. We determined that the BAC-transgene does not affect the phenotype of $D1R^{-/-}$ mice. BAC-$D1R^{-/-}$ mice exhibited hyperlocomotion and impaired motor coordination (Supplementary Fig. 3) as described for $D1R^{-/-}$.[29,30,32] Tyrosine hydroxylase protein-ir is similar in WT, BAC-$D1R^{-/-}$, and $D1R^{-/-}$ naïve mice, confirming previous data,[33] and 6-OHDA lesions similarly affected striatal TH levels in both types of mice. The FosB response after L-dopa treatment was blunted in BAC-$D1R^{-/-}$ as in $D1R^{-/-}$ mice[19] (Supplementary Fig. 3).

Inactivation of D1R Increases Spiking in d-SPNs

Dopamine receptors have opposite roles in regulating the spiking of SPNs: D1R increases the firing rate of d-SPNs, whereas D2R reduces the rate in i-SPNs,[39] although Lemos and colleagues[40] saw no change in i-SPN excitability after acute D2R agonist. However, the lack of activation of both receptors in PD increases the spiking in both SPNs.[12,13,16,17] To determine the exact role of D1R in the firing rate of SPNs, we studied the frequency of the evoked action potentials in $D1R^{-/-}$ mice. In d-SPNs, the number of evoked action potentials increased in $D1R^{-/-}$ mice compared with WT mice at all tested intensities (Fig. 1A), as in PD mouse models.[12,13] This increased firing rate correlates with the decreased rheobase of d-SPNs compared to WT (Table 1). Other action potential properties (threshold, amplitude, or amplitude after the hyperpolarization period) and passive membrane properties in $D1R^{-/-}$ mice are similar to WT mice (Table 1). In contrast, the inactivation of D1R in i-SPNs does not affect the number of evoked action potentials (Fig. 1A) or their intrinsic membrane properties (Table 1). In WT mice, d-SPNs show fewer spikes than i-SPNs (Fig. 1A), as shown previously.[16,41-46] However, the specific increase in the d-SPNs firing rate induced by D1R inactivation drives both SPNs to exhibit similar spiking (Fig. 1A).

In PD models, using somatic stimulation, the lack of dopamine reduces the threshold for evoked action potentials specifically in d-SPNs.[12] We studied whether D1R inactivation reproduces this specific synaptic facilitation by evoking backpropagated action potentials in the soma with increasing stimulation intensities. The presynaptic fibers were stimulated with an electrode in the white matter. d-SPNs in $D1R^{-/-}$ mice had a lower spike threshold than in WT mice (Fig. 1B), and no change was observed in i-SPNs (Fig. 1B). This difference facilitates EPSP-spike generation in d-SPNs relative to i-SPNs.

Genetic Inactivation of D1R Reduces the Dendritic Tree in Both Types of SPNs

Severe lack of striatal dopamine reduces dendritic tree complexity in PD model animals.[10,11,47,48] To determine the role of D1R in these changes, we studied the impact of D1R genetic inactivation on dendritic arbor complexity in both types of SPNs. In $D1R^{-/-}$ mice, dendritic tree length is reduced in both d-SPNs and i-SPNs compared with WT (Fig. 2A) and is homogeneous along the dendritic arbor, shown by Sholl analysis. The number of nodes was not reduced in either type of SPNs (data not shown). The shrinkage is not attributed to the loss of primary dendrites because d-SPNs and i-SPNs of $D1R^{-/-}$ mice have the same number of primary dendrites as WT with a similar maximum dendritic order (data not shown).

To compare the effect of D1R genetic inactivation with acute dopamine depletion, we treated a group of WT mice with reserpine. In $D1R^{-/-}$ and reserpine-treated WT mice, the total dendritic length was similar in d-SPNs (1966.37 $\mu m \pm 150.11$ in $D1R^{-/-}$ and 1796.88 $\mu m \pm 264.53$ in WT-reserpine mice; data not shown) and i-SPNs (1989.02 $\mu m \pm 156.02$ in $D1R^{-/-}$ and 1640.88 $\mu m \pm 62.51$ in reserpinized WT mice; data not shown). Therefore, the genetic inactivation of D1R reduces the complexity of dendritic arborization in both types of SPNs similarly to acute dopamine depletion.

Genetic Inactivation of D1R Reduces Spine Density in Both Types of SPNs

Lack of dopamine causes the loss of spines in both types of SPNs.[11-14,48,49] We studied the role of D1R in these changes and whether they affect 1 or both SPNs types. In $D1R^{-/-}$ mice, the spine density of d-SPNs is reduced by 34% relative to WT (Fig. 2B), with 5.37 ± 0.19 and 8.14 ± 0.56 spines per 10 μm in $D1R^{-/-}$ and WT mice, respectively. This reduction is observed in proximal and distal dendritic parts, demonstrated by Sholl analysis (Fig. 2). Spine density is also reduced in i-SPNs, although at a much lower reduction (14%) than in d-SPNs (Fig. 2C), with 6.66 ± 0.18 spines per 10 μm between 45 to 120 μm from soma in $D1R^{-/-}$ versus 7.77 ± 0.41 in WT mice (Fig. 2B,C). Therefore, D1Rs are primarily involved in spine maintenance in d-SPNs, but also play a significant role in i-SPNs.

Striatal Glutamate Release Does Not Change in $D1R^{-/-}$ Mice

The spine loss observed in PD could represent a neuroprotective mechanism of SPNs to reduce synaptic contacts in response to elevated glutamate in the striatum.[50] Because $D1R^{-/-}$ mice exhibit an increased glutamate/glutamine cycle,[32] we determined if spine loss in i-SPNs was caused by increased striatal glutamatergic synaptic transmission. We studied synaptic glutamate release by measuring the paired-pulse ratio (PPR) and the spontaneous EPSCs in the presence of picrotoxin (100 μM) to avoid the influence of GABAergic transmission. For PPR, we evoked 2 consecutive EPSCs 100 milliseconds apart. In WT mice, i-SPNs exhibit higher facilitation than d-SPNs (Supplementary Fig. 4A) as previously shown[43]; genetic D1R inactivation did not modify the PPR in i-SPNs (Supplementary Fig. 4A; 1.35 ± 0.05 and 1.23 ± 0.08 for $D1R^{-/-}$ and WT, respectively) or d-SPNs (1.04 ± 0.07 and 0.98 ± 0.08 for $D1R^{-/-}$ and WT, respectively; Supplementary Fig. 4A). This indicates that presynaptic glutamatergic release does not change in $D1R^{-/-}$ mice. An analysis of sEPSC at a

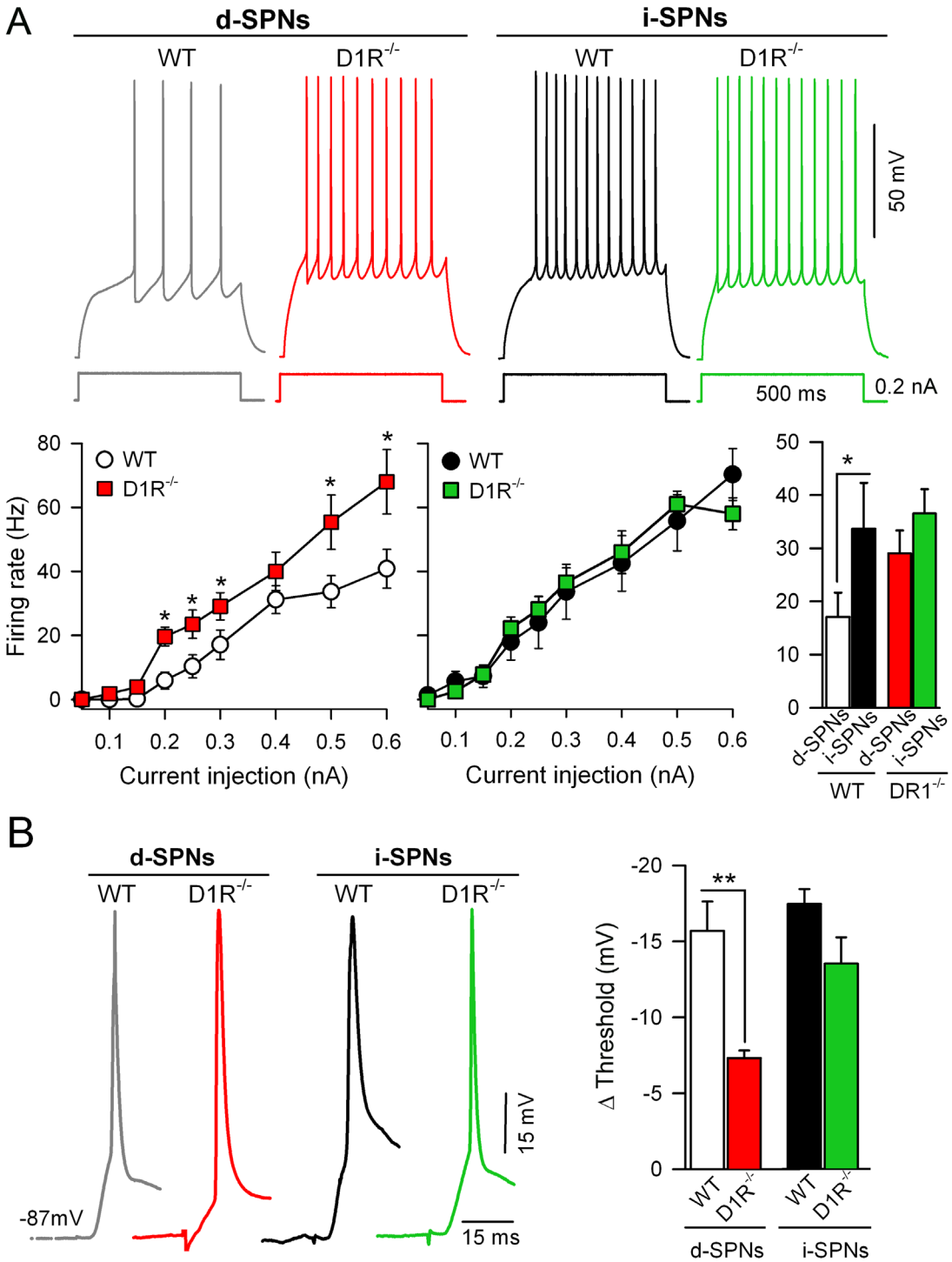

FIG. 1. Genetic D1 receptor inactivation increases the excitability of d-SPNs. (**A**) Representative whole-cell recordings showing SPNs recorded at 0.2 nA (top). Frequency of action potential evoked with depolarizing current in d-SPNs and i-SPNs (bottom). Histograms show the mean firing rate at 0.3 nA to facilitate the comparison between d-SPNs with i-SPNs. *$P < 0.05$; 2-way analysis of variance following Bonferroni posttest. (**B**) Illustrative traces of action potentials evoked by synaptic stimulation in WT and $D1R^{-/-}$ mice. Summary of changes in the threshold of synaptically evoked action potentials. **$P < 0.005$; 1-way analysis of variance following Bonferroni posttest. $D1R^{-/-}$, knockout D1 receptor mice; d-SPNs, direct-pathway striatal projection neurons; i-SPNs, indirect-pathway striatal projection neurons; SPNs, striatal projection neurons; WT, wild type. [Color figure can be viewed at wileyonlinelibrary.com]

SPN membrane potential of −70 mV showed no differences in amplitude, frequency, or probability of interevent interval between d-SPNs and i-SPNs in WT mice, as previously found.[51] No changes were caused by D1R inactivation (Supplementary Fig. 4B), indicating no changes in glutamatergic synaptic release in $D1R^{-/-}$ mice.

TABLE 1. Intrinsic membrane properties of SPNs from WT and D1R$^{-/-}$ mice

	d-SPNs				i-SPNs			
	WT		D1R$^{-/-}$		WT		D1R$^{-/-}$	
Membrane Properties	Naïve (12)	Naïve (14)	6-OHDA (5)	6-OHDA + L-dopa (8)	Naïve (12)	Naïve (11)	6-OHDA (4)	6-OHDA + L-dopa (6)
Passive								
RMP (mV)	−82.80 ± 1.37	−83.92 ± 1.55	−89.64 ± 2.10	−82.86 ± 2.29	−82.17 ± 1.30	−85.46 ± 1.22	−89.78 ± 3.27	−86.17 ± 3.31
Rin (mΩ)	53.61 ± 3.09	49.47 ± 3.53	56.12 ± 4.22	48.71 ± 3.44	51.00 ± 3.19	53.25 ± 2.54	55.61 ± 4.51	56.41 ± 4.73
Active								
Rheobase (nA)	0.22 ± 0.02	0.17 ± 0.02[a]	0.16 ± 0.01	0.18 ± 0.02	0.15 ± 0.02[b]	0.14 ± 0.01	0.14 ± 0.02	0.20 ± 0.02
AP threshold (mV)	−44.38 ± 1.01	−44.94 ± 1.42	−46.15 ± 4.96	−48.07 ± 4.39	−41.73 ± 1.35	−44.93 ± 0.94	−44.54 ± 1.03	−50.39 ± 1.53[c]
AP amplitude (mV)	80.01 ± 2.38	82.15 ± 1.59	86.40 ± 1.56	87.73 ± 1.30	82.57 ± 1.38	83.57 ± 1.53	86.94 ± 1.58	86.80 ± 1.10
AHP amplitude (mV)	−13.97 ± 0.73	−10.50 ± 1.66	−9.58 ± 2.20	−10.37 ± 1.44	−10.22 ± 2.49	−12.17 ± 0.91	−13.07 ± 3.24	−10.41 ± 1.51

Data are presented as mean ± standard error of the mean. The number of SPNs-recorded in each condition is expressed in parentheses following the condition. One-way analysis of variance or Kruskal-Wallis analysis.

[a] $P < 0.05$ WT versus D1R$^{-/-}$ in each type of SPN.

[b] d-SPN versus i-SPN in WT mice.

[c] $P < 0.05$ D1R$^{-/-}$ naive versus D1R$^{-/-}$ 6-OHDA or 6-OHDA L-dopa in each type of SPN.

Abbreviations: SPNs, striatal projection neurons; WT, wild type; D1R$^{-/-}$, knockout D1 receptor mice; d-SPNs, direct-pathway striatal projection neurons; i-SPNs, indirect-pathway striatal projection neurons; L-dopa, levodopa; 6-OHDA, 6-hydroxydopamine; RMP, resting membrane potential; Rin, internal resistance; AP, action potential; AHP, after-hyperpolarization period.

Dopamine Depletion Does Not Change Dendritic Length in D1R$^{-/-}$ Mice

Inactivation of D1R reduces the dendritic length of both types of SPNs. To understand how dopaminergic transmission affects the arborization of SPNs, we determined if dopamine acts via D1R by assessing if dopamine depletion further reduces dendritic length in D1R$^{-/-}$ mice. We studied the morphology of SPNs in 6-OHDA-lesioned D1R$^{-/-}$ mice (Supplementary Table 1) in the totally denervated striatum. The complexity of the dendritic arbor did not change after the lesion; there were no changes in the Sholl analysis of the dendritic length in either d-SPNs or i-SPNs (Fig. 3A). Chronic administration of L-dopa, the main treatment for PD, does not alter the dendritic tree of SPNs in 6-OHDA-lesioned D1R$^{-/-}$ mice. Therefore, a severe lack of dopamine does not alter dendritic arborization more than that induced by D1R inactivation, and subsequent D2R stimulation with L-dopa in D1R$^{-/-}$ does not produce additional changes.

Dopamine Depletion Does Not Alter the Spine Loss Induced in d-SPNs by D1R Inactivation, but Potentiates This Loss in i-SPNs

We next studied if the spine loss induced by D1R inactivation is further potentiated by dopamine depletion. In d-SPNs, the lack of dopamine in 6-OHDA-lesioned D1R$^{-/-}$ mice does not further reduce the spine density compared with naïve D1R$^{-/-}$ mice (5.26 ± 0.26 vs. 5.37 ± 0.19 spines per 10 μm in 6-OHDA-lesioned and naïve D1R$^{-/-}$ mice, respectively; Fig. 3B). Therefore, inactivation of D1R is sufficient to reduce the spine density in d-SPNs. However, in i-SPNs, the spine loss induced by D1R inactivation is not as robust as in d-SPNs, and dopamine depletion further decreases spine density (Fig. 3B) compared with naïve D1R$^{-/-}$ mice. This spine loss was observed in proximal and distal dendritic parts, shown by Sholl analysis, is similar to that observed in WT mice treated with 6-OHDA or treated with reserpine[11-12] o with reserpine (4.61 ± 0.28 vs. 4.96 ± 0.56 spines/10 μm, in 6-OHDA-lesioned D1R$^{-/-}$ and reserpinized WT, respectively). Therefore, in d-SPNs, the impact of D1R inactivation is similar to dopamine depletion, but in i-SPNs, the effect of D1R on spine density is potentiated by dopamine depletion.

In D1R$^{-/-}$ Mice, L-dopa Treatment Restores the Spine Loss Induced by Lesions in i-SPNs, but Not in d-SPNs

L-dopa treatment in PD animals selectively restores spine density in i-SPNs.[10-13] In D1R$^{-/-}$ mice, L-dopa does not change spine density in d-SPNs (Fig. 3B) and restores the spine loss in i-SPNs (4.61 ± 0.28 vs.

6.68 ± 0.22 spines/10 μm in D1R$^{-/-}$ lesioned mice treated with saline or L-dopa, respectively), but it is unable to restore the spine loss caused by D1R inactivation (Fig. 3B). Notably, the spine density recovery induced by L-dopa in i-SPNs in lesioned D1R$^{-/-}$ mice does not reach the density values observed in WT-lesioned mice treated with L-dopa (6.68 ± 0.22 vs. 7.59 ± 0.40 spines/10 μm in D1R$^{-/-}$ vs WT mice; $P < 0.05$ 1-way ANOVA), but density recovers to that observed in D1R$^{-/-}$ naïve mice (Fig. 3B). The spine

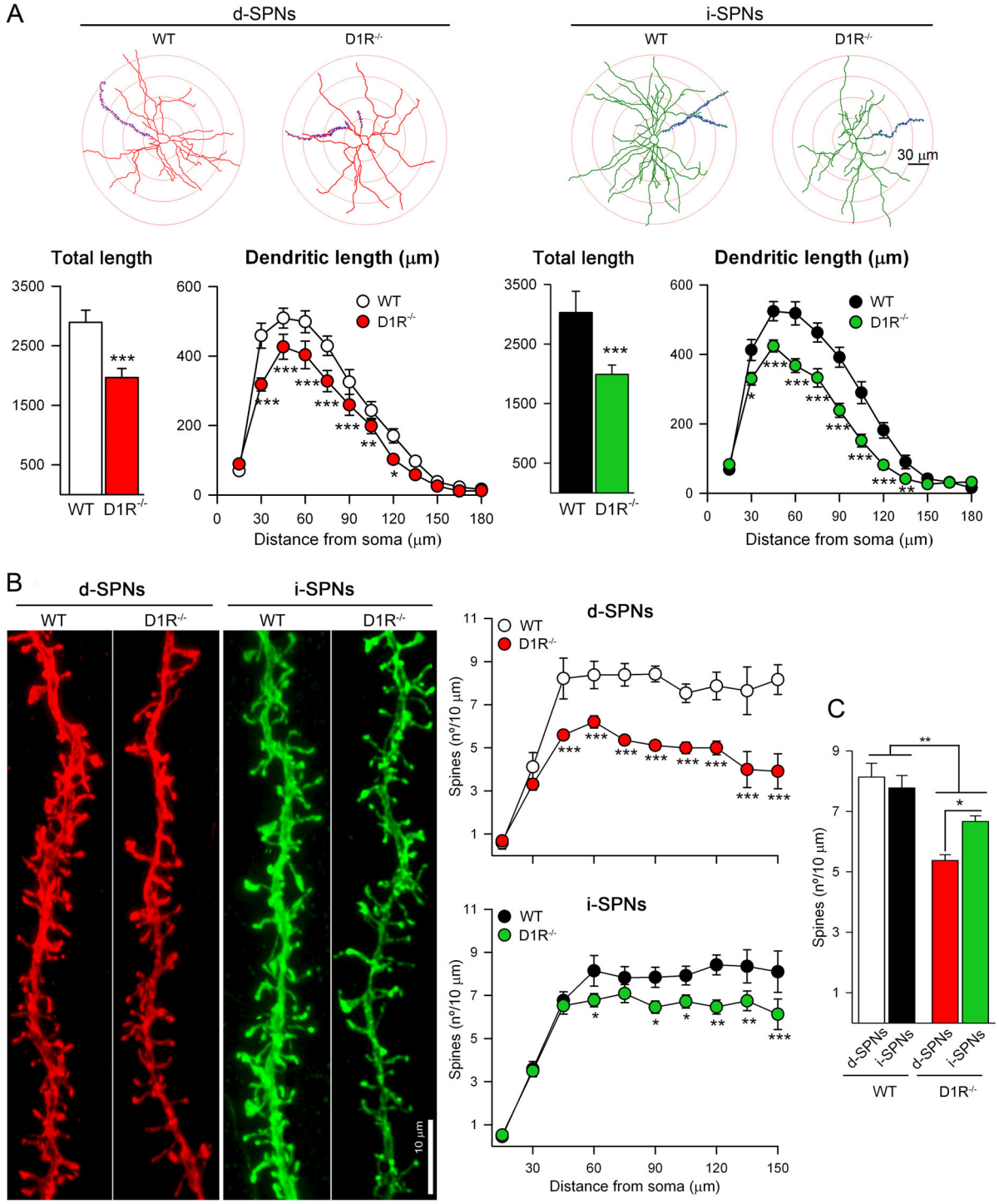

FIG. 2. Genetic D1 receptor inactivation reduces the dendritic tree complex and the spine density in both types of SPNs. (**A**) Illustrations of Sholl analysis of SPNs (top). Total length and Sholl analysis of the total dendritic length of d-SPNs and i-SPNs (bottom). (**B**) Representative confocal images of dendrites of SPNs and Sholl analysis of the spine density of SPNs. (**C**) Average of spine density (45–120 μm from the soma) to facilitate the comparison between d-SPNs with i-SPNs. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ WT versus D1R$^{-/-}$; 2-way analysis of variance following Bonferroni posttest. D1R$^{-/-}$, knockout D1 receptor mice; d-SPNs, direct-pathway striatal projection neurons; i-SPNs, indirect-pathway striatal projection neurons; SPNs, striatal projection neurons; WT, wild type. [Color figure can be viewed at wileyonlinelibrary.com]

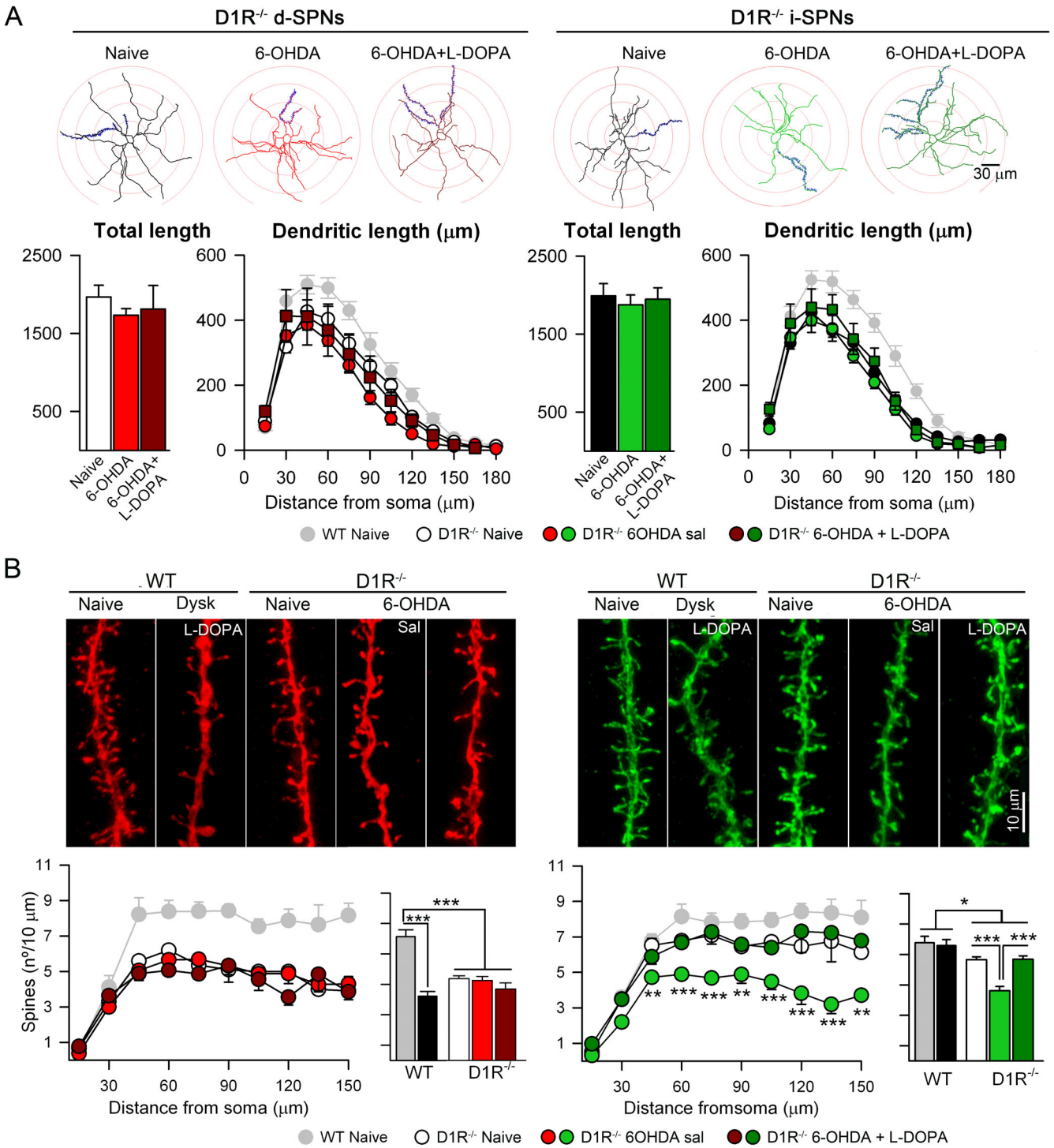

FIG. 3. The spine density of i-SPNs of D1R$^{-/-}$ mice decreases with dopamine depletion and recovers after L-dopa treatment. (**A**) Illustrations of Sholl analysis of SPNs in D1R$^{-/-}$ mice in each experimental condition (top). Total length and Sholl analysis of the total dendritic length of d-SPNs (left) and i-SPNs (right) in naïve or 6-OHDA-lesioned D1R$^{-/-}$ mice treated with saline or L-dopa. (**B**) Representative confocal images of dendrites of SPNs in naïve, lesion, and lesion plus L-dopa D1R$^{-/-}$ mice. Sholl analysis of the spine density of SPNs. Histograms show the average spine density between 45 to 120 µm from soma in naïve and dyskinetic WT mice in comparison to naïve and 6-OHDA-lesioned D1R$^{-/-}$ mice treated with saline or L-dopa. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; 2-way analysis of variance following Bonferroni posttest. Note: WT mice (gray trace) and naïve D1R$^{-/-}$ mice (open circles) shown in Figure 2 are included to facilitate the comparison. 6-OHDA, 6-hydroxydopamine; D1R$^{-/-}$, knockout D1 receptor mice; d-SPNs, direct-pathway striatal projection neurons; i-SPNs, indirect-pathway striatal projection neurons; L-dopa, levodopa; SPNs, striatal projection neurons; WT, wild type. [Color figure can be viewed at wileyonlinelibrary.com]

recovery is not attributed to L-dopa treatment alone because in nonlesioned D1R$^{-/-}$ and WT mice treated with L-dopa for 15 days, spine density does not change in either d-SPNs or i-SPNs (data not shown). These results indicate that in i-SPNs, D1R, and D2R help regulate the spine maintenance.

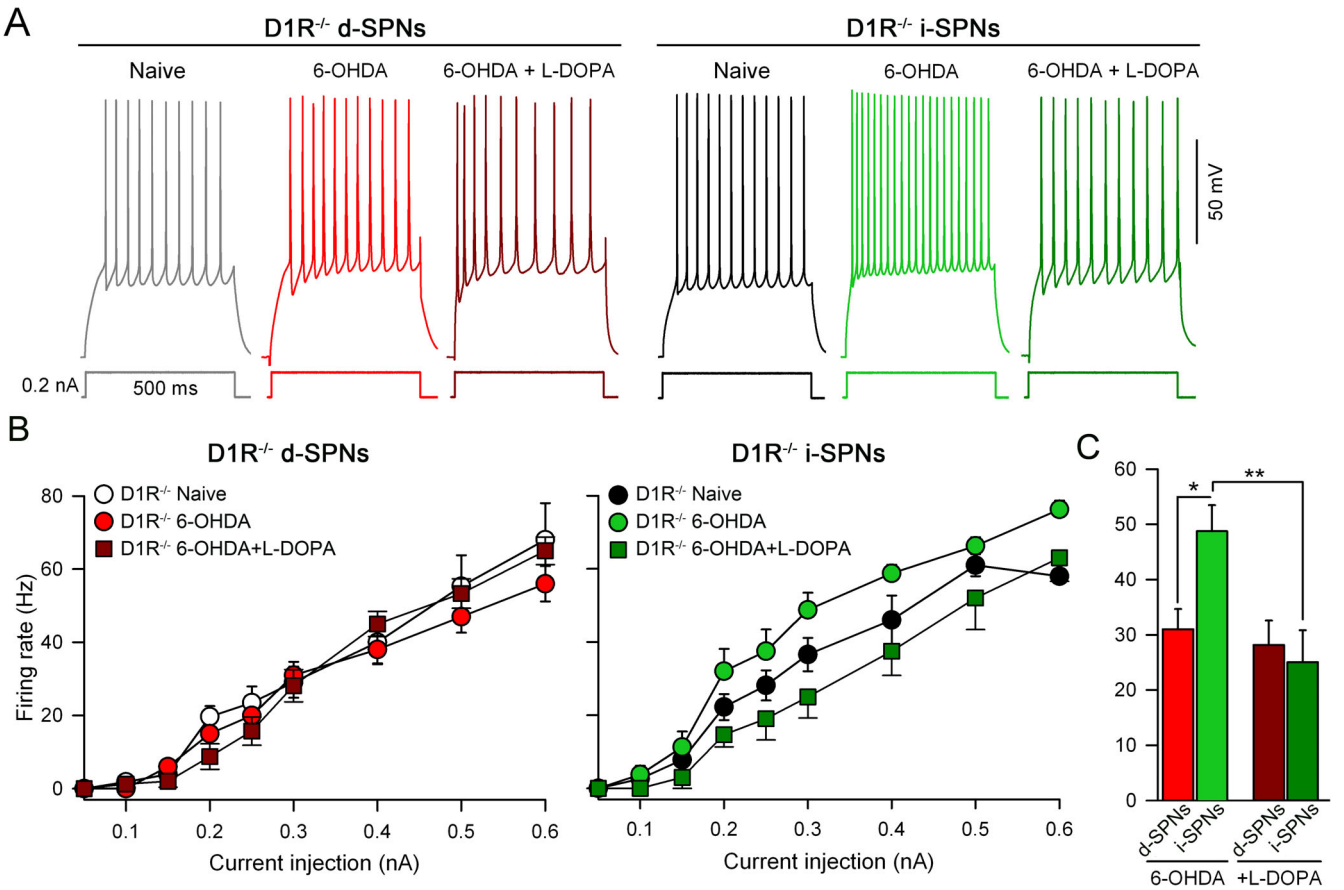

FIG. 4. L-dopa restores the hyperexcitability produced by dopamine depletion in i-SPNs in D1R$^{-/-}$ mice. (**A**) Representative whole-cell recordings showing SPNs in D1R$^{-/-}$ mice recorded at 0.2 nA. (**B**) Frequency of the action potential evoked with depolarizing current in d-SPNs and i-SPNs. Note: The values of naïve D1R$^{-/-}$ mice shown in Figure 1 are included to facilitate the comparison. (**C**) Histograms show the mean firing rate at 0.3 nA to facilitate the comparison between d-SPNs with i-SPNs in 6-OHDA-lesioned D1R$^{-/-}$ mice treated with saline or L-dopa. *$P < 0.05$, **$P < 0.005$; 2-way analysis of variance following Bonferroni posttest. 6-OHDA, 6-hydroxydopamine; D1R$^{-/-}$, knockout D1 receptor mice; d-SPNs, direct-pathway striatal projection neurons; i-SPNs, indirect-pathway striatal projection neurons; L-dopa, levodopa; SPNs, striatal projection neurons. [Color figure can be viewed at wileyonlinelibrary.com]

L-dopa Treatment Reverses the Hyperexcitability in i-SPNs Caused by Dopamine Depletion

Chronic L-dopa induces an imbalance between the intrinsic excitability of the 2 types of SPNs in 6-OHDA-lesioned mice, favoring d-SPNs.[10,12,13] We measured the firing rate in 6-OHDA-lesioned D1R$^{-/-}$ mice treated with L-dopa. In d-SPNs, the firing rate did not change after L-dopa (Fig. 4A). The passive membrane properties and action potential properties are similar in d-SPNs in lesioned mice and naïve D1R$^{-/-}$ mice (Table 1). However, in i-SPNs the firing rate is higher in lesioned mice compared with naïve D1R$^{-/-}$ mice at all tested intensities ($F_{2,127} = 17.39$; $P < 0.01$, 2-way ANOVA; Fig. 4), similar to observations in parkinsonian WT mice.[12,13] After L-dopa treatment, the firing rate reverts to naïve values (Fig. 4), overlapping with the slight increase of the rheobase, despite the threshold decrease of the action potential (Table 1). In lesioned D1R$^{-/-}$ mice, the spiking is lower in d-SPNs than in i-SPNs (Fig. 4C; $P < 0.05$ 2-way ANOVA). After L-dopa, both SPNs exhibit a similar firing rate (Fig. 4C), unlike dyskinetic WT animals, whose d-SPNs exhibit more spikes than i-SPNs.[12,13] Therefore, D1R inactivation blocks the increased firing rate induced in d-SPNs by L-dopa, matching the firing rate observed in i-SPNs.

Discussion

Our goal was to unravel the role of dopamine D1 receptors in the electrophysiological and structural plasticity of SPNs. In d-SPNs, genetic inactivation of D1R reduces dendritic tree length and spine density and increases firing rate, similar to changes caused by dopamine depletion in WT animals. Remarkably, D1R inactivation also affects i-SPNs, reducing dendritic tree complexity and spine density, although the spine loss is less severe than in d-SPNs and the firing rate of i-SPNs is unchanged. In D1R$^{-/-}$ mice, dopamine depletion does not further change d-SPNs, but in i-SPNs,

dopamine depletion increases spine loss and spiking. Dopamine replacement does not affect d-SPNs, but spine loss and spiking induced by the lesions are recovered in i-SPNs. ●

D1R Inactivation Reduces Spine Density and Dendritic Arbor Complexity in SPNs

Dopamine is essential for the morphological maintenance of SPNs; in human patients with PD and animal models, a lack of dopamine reduces dendritic tree complexity and spine density.[8,24,52] The specific role of D1R on the dendritic arbor of d-SPNs and i-SPNs has not been explored. We demonstrated that genetic inactivation of D1R and dopamine depletion similarly alter the dendritic length and spine density of d-SPNs. Although the exact mechanisms are unclear, neonatal P1-2 SPNs treated with D1R or D2R agonists or cocultured with mesencephalic dopaminergic or cortical neurons increase the formation and maturation of dendritic spines.[53-55] In addition, studies in neonates (P8-13) showed that in d-SPNs, D1R activation increases spine density, accelerates its formation,[27] and enlarges the spines when coactivated with glutamatergic inputs,[28] suggesting a cooperative role of D1R and glutamate during d-SPN spine development.

Genetic inactivation of D1R also reduces the length of the dendritic tree and spine density of i-SPNs, even though these neurons only express D2R. The reduction of the dendritic arbor in i-SPNs is similar in naïve and 6-OHDA-lesioned D1R$^{-/-}$ mice, which could indicate that D1R is important for i-SPN dendritic length. Previous studies have implicated D2R in the maintenance of the dendritic arbor of SPNs,[56] so the lack of D1R during striatal development could occlude the additional shrinkage induced by the toxin, masking the role of D2R in adult mice. However, the precise mechanisms underlying D1R inactivation-induced spine reduction in i-SPNs remain unknown. One likely mechanism that could explain spine decrease in both SPNs is a reduced corticostriatal glutamatergic transmission through the basal ganglia circuit caused by D1R inactivation. Indeed, in P14-15 mice, silencing γ-Aminobutyric acid release in d-SPNs, a downstream effect of D1R inactivation, reduces spine density in d-SPNs and i-SPNs by reducing corticostriatal glutamate release at the circuit level.[25] Although we do not detect changes in synaptic glutamate release in D1R$^{-/-}$ mice, we cannot exclude a decreased glutamatergic innervation as a consequence of D1R inactivation, and in fact, we favor this interpretation. In addition, it is also possible that the spine loss we observed using our constitutive D1R$^{-/-}$ mice is attributed to compensatory mechanisms during development. However, in Pitx3$^{-/-}$ mice showing a severe lack of dopamine neurons, dorsal striatal SPNs develop without dopamine signaling and nevertheless respond to a dopaminergic stimulus (L-dopa) in the same way as SPNs lesioned with 6-OHDA at adulthood.[13] This observation argues against a developmental deficit and supports the key role of D1R in the morphological maintenance of SPNs. Further experiments are needed using conditional-D1R$^{-/-}$ mice to rule out this possibility.

Reserpine treatment induces a severe parkinsonian syndrome and causes spine pruning affecting both types of SPNs, as in PD models and agreeing with our studies[11-13] and others,[8,14,57] but disagreeing with some others.[7,10] Recently, Graves and Surmeier[49] found spine loss in d-SPNs longer after lesion, although other groups find it earlier.[57]

Chronic L-dopa treatment selectively recovers the 6-OHDA-induced loss of dendritic spines in i-SPNs.[10-13] We found that L-dopa induces no changes in the spine density of d-SPNs in lesioned-D1$^{-/-}$ mice, similar to WT animals. L-dopa rescues the spine loss caused by 6-OHDA in i-SPNs, but not the loss caused by D1R inactivation. This effect suggests that D1R and D2R cooperate in the maintenance and formation of spines in i-SPNs, agreeing with previous results.[53,55]

Excitability of SPNs Is Imbalanced in D1$^{-/-}$ Mice

Dendritic spines contribute to functional and plastic changes in brain networks. In PD, the loss of dendritic spines is compensated for by increasing the intrinsic excitability of SPNs.[12,13,16,17] Genetic inactivation of D1R reduces spine density, increases the firing rate, and decreases the threshold for action potentials induced by synaptic stimulation of d-SPNs, as in PD models. D2R overexpression decreases dendritic arbor length in both SPNs while increasing their excitability via potassium channels.[56] The intrinsic excitability does not change in i-SPNs, despite the reduction of spine density and the length of the dendritic tree, so higher atrophy may be necessary. At 50% spine loss, as in 6-OHDA-lesioned D1R$^{-/-}$ mice, the intrinsic excitability of i-SPNs increases. Alternatively, the activation of D2R maintains i-SPNs spiking in a physiological range in D1R$^{-/-}$ mice. The nigrostriatal pathway is not altered in D1R$^{-/-}$ mice, allowing dopamine, via D2R, to decrease the excitability of i-SPNs. The hyperexcitability of d-SPNs could block the increased firing rate of i-SPNs. The classic model proposing the relative independence of the striatal pathways has recently been revised. Electrophysiology and calcium-imaging data indicate functional interactions between d-SPNs and i-SPNs, which form neuronal clusters or ensembles during specific aspects of motor behavior,[58-69] either by direct intrastriatal influence (SPN-SPN) via interneurons, or multisynaptic connections with the motor cortex. Direct synaptic connections of d-SPNs to i-SPNs is supported by anatomical data

revealing that collaterals synapses from d-SPNs to i-SPNs are weak.[70,71] However, recent research has shown that these connections strongly inhibit the other SPNs when there are multiple activations.[72] In D1R$^{-/-}$ mice, the hyperexcitability of d-SPNs could be enough to block i-SPN hyperexcitability. Alternatively, increased spiking of d-SPNs observed in D1R$^{-/-}$ mice could activate striatal interneurons responsible for reducing the excitability of i-SPNs. Further experiments will resolve this issue because fast-spiking or cholinergic interneurons regulate the excitability of SPNs, and their activation is modified in PD.[57,73,74]

In physiological conditions, d-SPNs are less active than i-SPNs,[13,16,41-46] whereas in PD models, the spiking of both types of SPNs is similar.[12,13,16,17] The genetic inactivation of D1R increases the excitability of d-SPNs, driving both striatal pathway neurons to a similar activation state. Although D1R$^{-/-}$ mice lack dopamine, this does not alter the spiking in d-SPNs, the firing rate and the spine loss are increased in i-SPNs, and L-dopa treatment selectively recovers the spiking rate in i-SPNs, similarly activating both neurons. D1R$^{-/-}$ mice do not develop dyskinesia,[19] so L-dopa-induced dyskinesia could be related to the higher activation of d-SPNs in WT-lesioned mice.

In summary, dendritic arborization and excitability of d-SPNs are similarly altered by D1R deletion or by dopamine depletion; i-SPNs are slightly affected by D1R deletion but strongly affected by dopamine depletion. Dopamine replacement in 6-OHDA-lesioned D1R$^{-/-}$ mice partially restores spine density in i-SPNs, but not in d-SPNs. This highlights a pivotal role for D1R in the structural plasticity of both types of SPNs and reveals how D1R regulates synaptic transmission related to PD.

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Supporting Data

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